Beneficial Effects of Conversion From Cyclosporine to Azathioprine on Fibrinolysis in Renal Transplant Recipients

Marinus A. van den Dorpel, Arie J. Man in’t Veld, Marcel Levi, Jan Wouter ten Cate, Willem Weimar

Abstract—Cyclosporin A (CsA) has been implicated as one of the factors contributing to the high cardiovascular morbidity and mortality after renal transplantation. This may be mediated by either a high prevalence of conventional risk factors for atherosclerosis, such as hypertension, hypercholesterolemia, and diabetes mellitus, or by impairment of the fibrinolytic activity evoked by CsA, possibly through interference with prostanoid metabolism. We therefore assessed the impact of conversion of CsA to azathioprine immunosuppressive treatment on parameters of fibrinolytic activity and plasma concentration of the prostanoids prostaglandin E2 and thromboxane B2 in 18 stable renal transplant recipients. During CsA, mean arterial pressure and serum creatinine were significantly higher than during azathioprine (116±15 mm Hg versus 106±13 mm Hg, P=0.0003; and 147±34 μmol/L versus 127±35 μmol/L, P=0.002; mean±SD). On conversion, the plasma tissue plasminogen activator activity increased from 1.2 (1.1 to 1.7; median, 95% CI) to 1.8 (1.6 to 2.0) IU/mL (P=0.011), without a significant change of the plasminogen activator antigen concentration. This was associated with a substantial decrease in plasminogen activator inhibitor-1 activity from 10.4 (8.5 to 16.7) to 6.4 (5.6 to 9.2) IU/mL (P=0.009). Furthermore, plasma levels of prostaglandin E2 and thromboxane B2 markedly decreased (from 9.7 [7.4 to 12.9] to 4.6 [4.3 to 8.1] pg/mL, P=0.006; and from 106.1 [91.7 to 214.2] to 70.2 [50.3 to 85.6] pg/mL, P=0.002, respectively). During CsA, but not azathioprine, plasma tissue plasminogen activator antigen and plasminogen activator inhibitor-1 levels correlated significantly with prostaglandin E2 (r=0.53, P=0.02; and r=0.60, P=0.008, respectively), and thromboxane B2 (r=0.75, P=0.0001; and r=0.77, P=0.0001, respectively) levels. In conclusion, CsA induced substantial impairment of fibrinolytic activity, which recovered after conversion to azathioprine. The impaired fibrinolysis observed during CsA treatment may be caused by modulation of eicosanoid production or metabolism in vascular endothelial cells and possibly contributes to the high incidence of cardiovascular disease after kidney transplantation. (Arterioscler Thromb Vasc Biol. 1999;19:1555-1558.)

Key Words: kidney transplantation ■ cyclosporin A ■ fibrinolysis ■ prostanoids

In renal transplant recipients the morbidity and mortality caused by atherosclerosis are substantially higher compared with the normal population.1 Cardiovascular disease accounts for up to 40% of deaths after renal transplantation, and there is increasing evidence that patients who are treated with cyclosporin A (CsA) are at high risk.2–4 Hypertension and hyperlipidemia are undoubtedly involved; however, disturbances in the fibrinolytic system appear to play an important role in the pathogenesis of atherosclerosis and thrombosis as well.5–6 Impaired activity of the fibrinolytic system mostly results from excess plasminogen activator inhibitor-1 (PAI-1), and less commonly from reduced release of tissue or urokinase-type plasminogen activator, and has been associated with the use of the immunosuppressant CsA.7 Because of endothelial cell damage and toxicity, CsA has been shown to increase the plasma levels of PAI-1, which results in decreased fibrinolytic activity.8 CsA-induced disturbances in prostanoid metabolism may also be implicated in the pathogenesis of the reduced fibrinolytic activity.9 We therefore hypothesized that conversion from CsA to azathioprine (AZA) as long-term immunosuppressive therapy would potentially ameliorate fibrinolytic activity. In the first prospective controlled study with paired observations to address this issue, we compared several parameters of the fibrinolytic system in renal transplant patients during CsA and after conversion to AZA treatment to determine the reversibility of the possible harmful effects on the fibrinolytic axis of long-term CsA. Plasma levels of prostaglandin E2 (PGE2) and thromboxane B2 (TXB2) were determined to assess whether CsA-induced interference with prostanoid metabolism is associated with impaired fibrinolysis, as has been suggested by others.9

Methods

Patients

Patients were recruited among renal transplant recipients who had been enrolled in a prospective randomized clinical trial, which was designed to evaluate the effects of 2 different immunosuppressive
Effects of Cyclosporine and Azathioprine on Fibrinolysis

TABLE 1. Patient Characteristics

<table>
<thead>
<tr>
<th>CaA</th>
<th>AZA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male/female</td>
<td>16/3</td>
</tr>
<tr>
<td>Age, y</td>
<td>41±23</td>
</tr>
<tr>
<td>Time after transplantation, mo</td>
<td>24±5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.9±3.5</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>116±14</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL/min</td>
<td>50±14</td>
</tr>
<tr>
<td>Prednisone dose, mg/d</td>
<td>9.6±2.1</td>
</tr>
<tr>
<td>CaA dose, mg · kg⁻¹ · d⁻¹</td>
<td>5.5±1.7</td>
</tr>
<tr>
<td>AZA dose, mg · kg⁻¹ · d⁻¹</td>
<td>...</td>
</tr>
</tbody>
</table>

*P<0.05, CaA vs AZA, paired t test.

Statistics

All data are presented as median±SD, unless indicated otherwise. Paired Student’s t test and Wilcoxon’s signed rank test were used as appropriate for assessing the statistical significance of the differences between CaA and AZA therapy. The relationships between different parameters were calculated with Pearson’s correlation coefficient.

Results

Clinical Characteristics

During CaA, mean arterial pressure was significantly higher (116±15 mm Hg versus 106±13 mm Hg; mean±SD; P=0.0003), and serum creatinine was significantly higher (147±34 μmol/L versus 127±35 μmol/L; mean±SD; P=0.002), than during AZA. Body mass index did not change on conversion. The daily dose of prednisone was identical during both treatments (Table 1). No acute rejection episodes occurred between both examination sessions.

Fibrinolysis and Prostanoids

Conversion from CaA to AZA was followed by an increase in tPA activity from 1.2 (1.1 to 1.7) IU/mL to 1.8 (1.6 to 2.0) IU/mL (P=0.011; normal value, 1.7 [1.4 to 2.0] IU/mL). As shown in Figure 1A, the increase in tPA activity appeared to be caused by a reduction in PAI-1, as reflected by a decrease in plasma levels of PAI-1 antigen (Figure 1D) and associated activity (Figure 1C), and virtually unchanged levels of tPA antigen (Figure 1B). The slight decrease in tPA antigen levels can be readily explained by the fact that this assay also measures tPA-PAI-1 complexes, and will therefore be somewhat affected by decreasing PAI-1 levels. Plasma PAI-1 activity and antigen levels decreased from 10.4 (8.5 to 16.7) to 6.4 (5.6 to 9.2) IU/mL (P=0.009; normal, 7.7 [6.1 to 9.3] IU/mL), and from 13.4 (9.5 to 18.2) to 8.8 (7.2 to 11.3) ng/mL (P=0.016; normal, 7.3 [5.9 to 8.7] ng/mL), respectively. The enhancement of tPA activity resulted in an increase of plasmin formation, as reflected by an increase in PAP complex levels from 3.4 (3.0 to 5.8) to 5.2 (4.1 to 6.4) mmol/L (P=0.05; Table 2). Plasma concentrations of both PGE₂ and TXB₂ were significantly lower during AZA treatment (Table 2).

Correlation Between Prostanoids and Fibrinolysis Parameters

During CaA but not AZA treatment, we observed strong positive correlations between plasma levels of PAI-1 antigen and PGE₂ (r=0.60, P=0.008; Figure 2, top left) and also between PAI-1 antigen and TXB₂ (r=0.77, P=0.0001; Figure 2, top right). The plasma concentration of tPA antigen was positively correlated to...
plasma PGE₂ \( (r=0.53, P=0.02) \) and plasma TXB₂ \( (r=0.75, P=0.0001; \text{data not shown}) \). Interestingly, TXB₂ was negatively correlated to tPA activity \( (r=-0.70, P=0.001; \text{Figure 2, bottom left}) \), which suggests that TXB₂-induced elevation of PAI-1 inhibits tPA antigen. This suggestion was supported by the negative correlation between TXB₂ and PAP-complex levels \( (r=-0.64, P=0.004; \text{Figure 2, bottom right}) \).

**Discussion**

The main finding to emerge from our study is that in renal transplant recipients, the activity of the fibrinolytic system increases substantially after conversion from CsA to AZA. The reason for this improvement appears to be a decrease in plasma PAI-1 levels. During CsA, but not AZA, we also found strong correlations between plasma prostanoid levels and several components of the fibrinolytic system.

In a number of studies, impaired fibrinolysis has been associated with the development of acute atherothrombotic events.5,6 We found that PAI-1 activity was significantly higher during CsA, which resulted in a reduction of tPA activity and subsequent plasmin formation as reflected by decreased PAP complexes. This finding is in agreement with data from a cross-sectional study, showing that in a group of CsA-treated renal transplant recipients PAI-1 was higher than in AZA-treated transplant recipients.15 The magnitude of the increase of plasma PAI-1 we found during CsA is comparable to the difference in PAI-1 levels between patients with an uneventful course after myocardial infarction and patients who had reinfarction.6

The mechanism of this CsA-associated impairment of fibrinolysis is not clear. A defective release of tPA and PAI-1 on 1-deamino-8-D-arginine vasopressin (DDAVP) stimulation was reported in CsA-treated renal transplant recipients, although basal tPA and PAI-1 levels were similar to those of AZA-treated patients.16 The impaired DDAVP-induced release of tPA was restored by fish-oil administration, suggesting that a CsA-induced alteration in prostanoid metabolism may be related to the impairment of fibrinolysis.

This hypothesis is supported by our finding that plasma PGE₂ and TXB₂ decreased markedly after conversion. This is in accordance with other data showing that during CsA systemic and intrarenal prostaglandin synthesis is disturbed.17,18 In addition, it has been shown that changes in eicosanoid dynamics may significantly affect vessel wall-related fibrinolytic activity.9 The notion that alterations in prostanoid metabolism are involved in the attenuation of fibrinolytic activity during CsA is further corroborated by our finding that plasma PGE₂ was closely correlated to plasma tPA activity and PAI-1 antigen and activity levels. In addition, TXB₂ was strongly negatively correlated to tPA activity and PAP complex levels, and positively correlated to PAI-1 antigen and PAI-1

**TABLE 2. Plasma Concentrations of PAP Complexes and Prostanoids During CsA and AZA**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CsA</th>
<th>AZA</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP, nmol/L</td>
<td>3.4 (3.0–5.8)</td>
<td>5.2 (4.1–6.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>PGE₂, pg/mL</td>
<td>9.7 (7.4–12.9)</td>
<td>4.6 (4.3–8.1)</td>
<td>0.006</td>
</tr>
<tr>
<td>TXB₂ pg/mL</td>
<td>106.1 (91.7–214.2)</td>
<td>70.2 (50.3–85.6)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are given as median values (95% CI).

*Calculated by Wilcoxon’s signed rank test.

**Figure 1.** Box whisker plots of plasma tPA activity (tPA-Act; A), tPA antigen (tPA-Ag; B), PAI-1 activity (PAI-1-Act; C), and PAI-1 antigen (PAI-Ag; D) during CsA and AZA treatment. *Indicates \( P=0.010 \); #, not significant; †, \( P=0.009 \); and ‡, \( P=0.016 \); CsA vs AZA by Wilcoxon’s signed rank test.

**Figure 2.** Scatterplots and linear regression lines with 95% CI of relationship between plasma PGE₂ or TXB₂ and several components of the fibrinolytic cascade during CsA treatment.
activity. However, during AZA, no correlation between prostanoids and fibrinolytic parameters was present, which may suggest that after conversion the normal regulatory dynamics of fibrinolysis and prostaglandin synthesis was restored.

It is tempting to speculate about alternative explanations for the impairment of fibrinolysis during CsA. In vitro, oxidized LDL is a potent stimulator of the release of PAI-1 by cultured endothelial cells.23 Interestingly, we and others recently found that during CsA treatment, LDL is more oxidized, because of increased susceptibility to oxidation both in vivo and in vitro.20,21 Therefore, it is conceivable that oxidized LDL may enhance the release of PAI-1 in vivo, resulting in reduced fibrinolytic activity.

The decrease in PAI-1 activity may also be associated with the simultaneous fall in blood pressure after conversion.23 In a cross-sectional study of healthy young men, the incidence of hypofibrinolysis caused by increased PAI-1 activity was increased in hypertensive subjects.23 The association between hypertension and fibrinolytic deficiencies may be mediated by a third factor, eg, hyperinsulinemia or insulin resistance.24 Other studies have demonstrated significant correlations between hypertriglyceridemia (which was also present during CsA), increased body weight, and hypofibrinolysis caused by increased PAI-1.25 Finally, an alternative mechanism may proceed through the LDL-receptor-related protein-α/macroglobulin receptor, which is involved in the internalization and intracellular degradation of complexes of plasminogen activator and its inhibitor PAI-1.26 CsA may lead to functional impairment of this receptor, leading to decreased PAI-1 clearance and, consequently, higher PAI-1 plasma levels.

The fact that all patients were examined during CsA first may have influenced our study results. Because of the current practice to use CsA as the initial immunosuppressing drug, randomization to CsA or AZA first was not possible. Therefore, a temporal bias cannot be completely excluded, although the patients had a stable graft function and were on average 24 months after transplantation.

In summary, we found that in renal transplant recipients, conversion from CsA to AZA is accompanied by a substantial enhancement of fibrinolytic activity. Our findings may contribute to the pathogenetic basis for the high incidence of cardiovascular disease after organ transplantation, which has been associated with the long-term use of CsA.2–4 Whether the positive effects of long-term treatment with CsA on graft survival are outweighed by the negative effects on cardiovascular morbidity and mortality should be a topic for future studies.

Acknowledgment
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References
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